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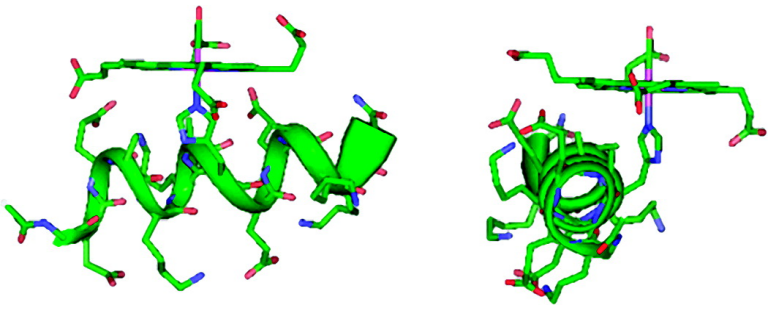

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NMR Structures of Peptide−Ru^{II}(Porphyrin) Complexes

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Heme proteins have a large array of functions, including electron transfer, oxygen transport, NO sensing, oxidant detoxification, and O_2 activation.[1] The protein provides one or two sites for ligation to the heme, which is also held in place either covalently (as in cytochrome c) or through multiple peripheral interactions (hydrophobic, hydrogen-bonding, or ion-pairing). The protein environment also fine-tunes the reactivity of the metalloporphyrin. As the study of metallporphyrins has grown, the use of synthetic analogues to mimic the wide range of heme protein behavior has improved as well. The mechanisms by which proteins specifically modify the reactivity of metalloporphyrins, however, are often still obscure. Designing minimal peptides as analogues of heme proteins can provide insight into these issues.[2,3] We have prepared a series of helical 15-mer peptides that bind strongly to metalloporphyrins and succeeded in solving their solution NMR structures.

The sequence of the 15-mers was designed with three criteria: (1) His was placed in the middle to provide for metal coordination; (2) hydrophobic residues were placed to interact favorably in an α-helical conformation with the hydrophobic surface of the porphyrin; and (3) hydrophilic residues (e.g., Glu and Lys) point away from the porphyrin and provide solubility (Figure 1). Finally, the sequence termini were capped to enhance helix dipole formation.[4] A list of the peptide sequences is shown in Table 1. Since His (which is located in the middle of the sequence) is a helix breaker, the apo peptides are random coils and not helical; once such a peptide binds to the heme, however, it changes conformation and shows substantial helicity. Moreover, the heme-peptide binding constants are about 3 orders of magnitude larger compared to His, primarily due to hydrophobic interaction between the peptide and porphyrin.[5]

The detailed NMR structures of these these minimal peptide−metalloporphyrin complexes have proved problematic for Fe(III), due both to its kinetic lability and to its paramagnetism. To overcome this problem, we prepared the CO adduct of peptide−Ru(II) porphyrin complexes. Coproporphyrin-I was chosen for its high symmetry. Ru(II) is diamagnetic, has low ligand exchange rates, and as the CO adduct, forms a 1:1 peptide complex.

Circular dichrosim (CD) experiments show that the 15-mer AAAKK (Table 1) was not initially helical, but after binding to Ru(copro)(CO), significant helicity results (Figure 1). Standard UV−vis titration[3] also shows that AAAKK binds strongly to Ru(copro)(CO), as shown in Table 1.

NOESY and TOCSY spectra[7] were collected to determine the NMR structure from the 1:1 peptide complex of Ru(copro)(CO) in aqueous solution. The NMR spectrum is nicely dispersed, indicating a well-defined structure. Figure 2 shows the NOESY spectra; the $d_{\alpha N(i,i+3)}$ NOE signals are visible for H8A5, A9A12, H8A11, and others (below threshold chosen for Figure 2). This is clear indication for α-helix secondary structure.

The d_{NN}, $d_{\alpha N(i,i+3)}$, $d_{\alpha\beta(i,i+3)}$ signals do not exist or are weak for residues near the termini of AAAKK, indicating that the α-helical

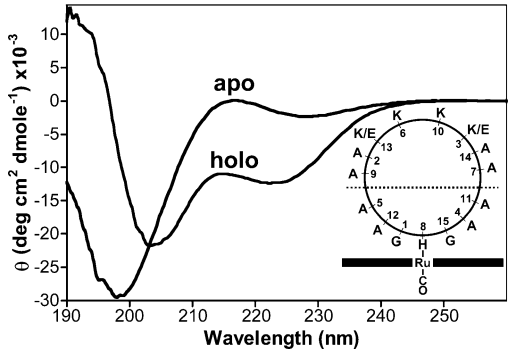

Figure 1. Circular dichroism spectra of AAAKK in the absence and presence of Ru(copro)(CO). The helix wheel diagram on the bottom left shows the peptide design (numbers indicate sequence).

Table 1. Properties of Peptides and Heme-Peptide Complexes[a]

peptide	K_b (mM^{-1})	apo peptide helicity %	holo peptide helicity %
AAAKK (water)	714	4.0	36
AAAKK (40% TFE)	872	37	41
AAAEK (water)	667	17	40

[a] Abbreviations: AAAKK, 15-mer peptide, Ac-GAKAAKAHAKAA-KAG-NH_2; AAAEK, 15-mer peptide, AcGAEAAKAHAKAAEAG-NH_2; Ru(copro)(CO), Ru^{II}(coproporphyrin-I-ato)(CO). Conditions: pH 7.6, 100 mM, potassium phosphate buffer. The percentage of helicity of the peptides may be approximated as %helicity = $\theta/[40000/(1-2.5/n)]$, when n equals the number of residues.[6]

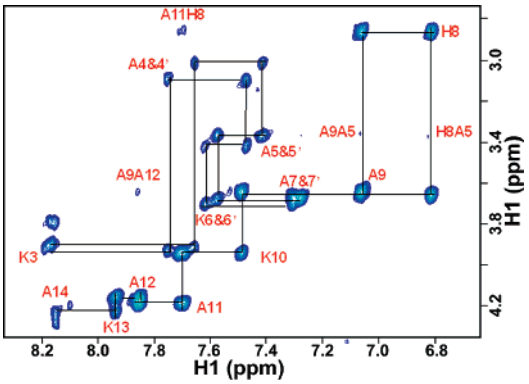

Figure 2. 2-D ^{1}H NOESY spectrum of Ru(copro)(CO) complexed with AAAKK. Because the Ru center of Ru(copro)(CO) is chiral, peptide binding results in two diasteriomers with two sets of signals.

structure is unstable at the ends of the peptide. We found that we could better stabilize the structure either by adding trifluoroethanol (TFE)[8] or by introducing salt bridges[9] into the peptide helix by replacing lysines K3 and K13 into glutamates. After adding TFE

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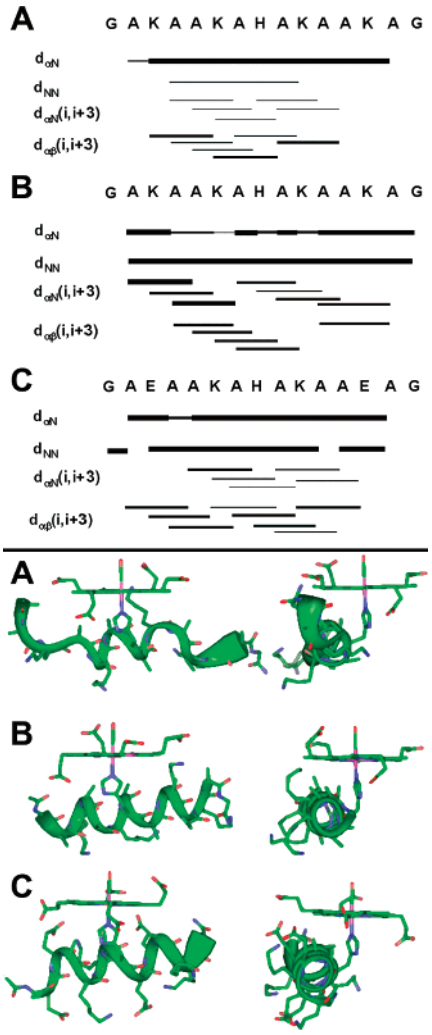

Figure 3. Summary of the sequential and long-range NOE and NMR solution structure. (Upper) NOE distances are divided into three types: strong (0−2.7 Å), medium (2.7−3.3 Å), and weak (3.3−5.0 Å). (A) Ru(copro)(CO) complexed with AAAKK in aqueous solution; (B) Ru(copro)(CO) complexed with AAAKK in 40% TFE aqueous solution; (C) Ru(copro)(CO) complexed with AAAEK in aqueous solution. (Lower) Consensus structures of A, B, and C, with views from the side and down the helix of each; rmsd (for all non-hydrogen atoms of the porphyrin-peptide complex) are 1.12, 0.72, and 0.78 Å, respectively, and residue-wise rmsd are mapped onto the thickness of the helix.

or introducing salt bridge, CD shows that the percent helicity increases for the peptide−porphyrin complex (Table 1). Two-dimensional NMR also shows much stronger d_{NN}, $d_{\alpha N(i,i+3)}$, $d_{\alpha\beta(i,i+3)}$ NOE signals, which also persist further toward the ends the peptide (Figure 3).

Finally, we used these NMR data to derive solution structures. The NOE distance retraints were determined by standard methods[7] and used to calculate structures. The porphyrin−peptide NOE constraints were not used because porphyrin has four meso protons, which makes it difficult to define their NOE distances. Special dihedral constraints are added to keep the His imidazole plane and porphyrin perpendicular as found in most heme proteins. By using the Insight-II Discover package (Accelrys, Inc.) with a modified form of CVFF force field (without nonbonded potentials, cf. Supporting Information for further details), 50 random structures are generated by using a simulated annealing protocol.[10] All of those structures converged to the same fold without significant unfavorable covalent or steric interactions; the mean structures are shown in Figure 3.

Interestingly, the structures show that the helix is tilted toward one side of the porphyrin. Because of this asymmetry, only alanines A4, A7, and A11 (but not A5, A9, and A12; cf. Figure 1 inset) form strong hydrophobic contacts with the porphyrin, which is further confirmed by the NOE signals between the porphyrin meso protons and β-protons of these residues. Such helix tilt is very common in heme proteins (including cytochrome c peroxidase, cytochrome c oxidase, cytochrome b_{562}, and myoglobin)[11] and is also present in our de novo designed cyclic-peptide heme complex.[3d] The helix tilt maximizes the heme-peptide hydrophobic contacts to make closer contact between the peptide and one-half of the porphyrin face. In some cases, the precise 3D structures of small peptide−porphyrin complexes may be difficult to define due to their intrinsic flexibility; even then, NMR data permits the characterization of both conformational preferences and dynamics.

In conclusion, we find that heme has not only a functional role in heme proteins, but also plays a profound structural role in the stabilization of secondary structure and helix orientation.

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Supporting Information Available: Structural statistics, NOESY and TOCSY spectra, NMR assignments, restraint file, and NMR structure in PDB format. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kadish, K. M.; Smith, K. M.; Guilard, R. *The Porphyrin Handbook*; Academic Press: London, 2000.

(2) (a) Lombardi, A.; Nastri, F.; Pavone, V. *Chem. Rev.* **2001**, *101*, 3165−89. (b) Gibney, B. R.; Dutton, P. L. *Adv. Inorg. Chem.* **2001**, *51*, 409−55. (c) DeGrado, W. F.; Summa, C. S.; Pavone, V.; Nastri, F.; Lombardi, A. *Ann. Rev. Biochem.* **1999**, *68*, 779−819. (d) Gibney, B. R.; Rabanal, F.; Dutton, P. L. *Curr. Opin. Chem. Biol.* **1997**, *1*, 537−42. (e) Liu, D.; Williamson, D. A.; Kennedy, M. L.; Williams, T. D.; Morton, M. M.; Benson, D. R. *J. Am. Chem. Soc.* **1999**, *121*, 11798−812. (f) Noy, D.; Discher, B. M.; Rubtsov, I. V.; Hochstrasser, R. A.; Dutton, P. L. *Biochemistry* **2005**, *44*, 12344−54. (g) Cochran, F. V.; Wu, S. P.; Wang, W.; Nanda, V.; Saven, J. G.; Therien, M. J.; DeGrado, W. F. *J. Am. Chem. Soc.* **2005**, *127*, 1346−7. (h) Ghirlanda, G.; Osyczka, A.; Liu, W.; Antolovich, M.; Smith, K. M.; Dutton, P. L.; Wand, A. J.; DeGrado, W. F. *J. Am. Chem. Soc.* **2004**, *126*, 8141−7.

(3) (a) Huffman, D. L.; Rosenblatt, M. M.; Suslick, K. S. *J. Am. Chem. Soc.* **1998**, *120*, 6183−4. (b) Huffman, D. L.; Suslick, K. S. *Inorg. Chem.* **2000**, *39*, 5418−9. (c) Rosenblatt, M. M.; Huffman, D. L.; Wang, X.; Remmer, H. A.; Suslick, K. S. *J. Am. Chem. Soc.* **2002**, *124*, 12394−5. (d) Rosenblatt, M. M.; Wang J. Y.; Suslick K. S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13140−5.

(4) Hol, W. G. J.; vanDuijnen, P. T.; Berendsen, H. J. C. *Nature* **1978**, *273*, 443−6.

(5) Chothia, C. *Nature* **1974**, *248*, 338−9.

(6) Chakrabartty, A.; Kortemme, T.; Padmanabhan, S.; Baldwin, R. L. *Biochemistry* **1993**, *32*, 5560−5.

(7) Wuthrich, K. *NMR of Proteins and Nucleic Acids;* John Wiley & Sons: Hoboken, NJ, 1986.

(8) Luo, P.; Baldwin, R. L. *Biochemistry* **1997**, *36*, 8413−21.

(9) Marqusee, S.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8898−902.

(10) Nilges, M.; Clore, G. M.; Gronenborn, A. M. *FEBS Lett.* **1987**, *219*, 11.

(11) Comparisons of our Ru-peptide can be made to structures of heme proteins using the angle defined by the helix axis to the metal center to porphyrin plane: RuAAAXK, 50°; myoglobin, 75°; cytochrome b562, 60°; and cytochrome c peroxidase, 40°. Further discussion is given in the Supporting Information.

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